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Draft Genome Sequences of Fungi Isolated from the International Space Station during the Microbial Tracking-2 Experiment

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ABSTRACT

As part of the Microbial Tracking-2 study, 94 fungal strains were isolated from surfaces on the International Space Station, and whole-genome sequences were assembled. Characterization of these draft genomes will allow evaluation of microgravity adaption, risks to human health and spacecraft functioning, and biotechnological applications of fungi.

ANNOUNCEMENT

Fungi are potential sources of nutrients and bioactive compounds during long-term spaceflight but also could affect astronaut health through both opportunistic infections and system biofouling (1, 2). As part of a study characterizing fungal responses to the space environment, we report the draft genomes of 94 fungal strains that were isolated from the International Space Station (ISS), representing 10 ascomycetous and 1 basidiomycetous species.

Aspergillus species are environmental fungi and opportunistic pathogens (3). Aspergillus unguis is a member of the ISS microbiome (4) and produces industrially important compounds (5). Aureobasidium pullulans is a black fungus that was previously isolated from the ISS water filtration system (6) and Mars mission spacecraft-associated surfaces (7).

Cladosporium species are dominant fungal contaminants in indoor air $(\underline{8}, \underline{9})$. Cladosporium sphaerospermum and Cladosporium cladosporioides were detected multiple time on the ISS, and their properties in microgravity were studied $(\underline{10}, \underline{11})$.

Fusarium veterinarium is a recently described species within the Fusarium oxysporum complex, the species of which are ubiquitous in soil, are known human/plant pathogens (12), and were isolated both from surfaces and from infected Zinnia hybrida plants aboard the ISS (13, 14). Fusarium annulatum, which has been isolated from plant and human tissues on Earth, has not been reported previously in space (15).

Penicillium species produce important bioactive compounds and can contaminate food and cause secondary infections (16). Previously detected on the ISS or Mir (2, 11) are *Penicillium citrinum*, a common soil and indoor species (17), *Penicillium rubens*, from which penicillin was isolated (18), and *Penicillium corylophilum*, which is commonly found in damp buildings (19). *Penicillium palitans*, which has been reported in cheese (20) and also in a wide range of habitats, including Antarctica (21), has not been reported previously in space.

Rhodotorula mucilaginosa is a ubiquitous environmental ($\underline{22}$) and human commensal yeast and opportunistic pathogen ($\underline{23}$) that is found in aquatic and built environments, including bathrooms and dishwashers ($\underline{24}$, $\underline{25}$). It is the most commonly isolated yeast on the ISS ($\underline{26-28}$).

Sample collection and fungal isolation steps were described elsewhere (26). For five flight missions, eight surfaces aboard the ISS were sampled with moistened polyester wipes (Table 1). Upon return to Earth, the wipes were agitated in sterile phosphate-buffered saline, which was concentrated using an InnovaPrep CP150 concentrating pipette, and suitable aliquots were spread onto nutrient media (Table 1). Fungal isolates were restreaked on potato-dextrose agar (PDA), and genomic DNA was extracted using the ZymoBIOMICS MagBead DNA kit according to the manufacturer's instructions. Whole-genome shotgun sequencing libraries were prepared with an Illumina Nextera DNA Flex library preparation kit (29) and were sequenced on the NovaSeq 6000 paired-end 2 × 150-bp platform with a S4 flow cell. After quality filtering and trimming with FastQC v0.11.7 (30) and fastp v0.20.0 (31), genomes were assembled using SPAdes v3.11.1 (32). Assembly quality was assessed with QUAST v5.0.2 (33). Fastp included screening for 512 adapters; otherwise, default settings were used for all steps.

TABLE 1.

Sampling locations, genetic loci used for taxonomic analysis, and WGS assembly quality for fungal species isolated from the ISS during the Microbial Tracking-2 mission

Sample name	Fungal species	Loci used for identification ^a	WGS accession no.	SRA accession no.	Medium and temperature _	Flig no
F6_8S_P_2A	Aspergillus unguis	benA, CaM	JAGUQD0000000000	SRR14342084	BA, 37°C	F6
F6_8S_P_4A	Aspergillus unguis	benA, CaM	<u>JAGUQC000000000</u>	SRR14342083	BA, 37°C	F6
F7_6S_YPD	Aureobasidium pullulans	ITS	<u>JAGUPW000000000</u>	SRR14342072	YPD, 25°C	F7
F7_5S_YPD	Aureobasidium pullulans	ITS	<u>JAGUPX000000000</u>	SRR14342073	YPD, 25°C	F7
F7_2S_YPD	Aureobasidium pullulans	ITS	JAGUPY0000000000	SRR14342074	YPD, 25°C	F7
F7_1S_YPD	Aureobasidium pullulans	ITS	JAGUQA000000000	SRR14342076	YPD, 25°C	F7
F7_2A_YPD	Aureobasidium pullulans	ITS	JAGUPZ0000000000	SRR14342075	YPD, 25°C	F7
F7_1A_YPD	Aureobasidium pullulans	ITS	JAGUQB000000000	SRR14342077	YPD, 25°C	F7
F6_1S_B_1B	Aureobasidium pullulans	ITS	<u>JAGUQJ000000000</u>	SRR14342071	R2A, 25°C	F6
F6_1S_P_3A	Aureobasidium pullulans	ITS	<u>JAGUQI000000000</u>	SRR14342125	BA, 37°C	F6
F6_4S_B_1	Aureobasidium pullulans	ITS	<u>JAGUQE000000000</u>	SRR14342118	R2A, 25°C	F6
F6_3S_1A_F	Aureobasidium pullulans	ITS	JAGUQH000000000	SRR14342124	PDA, 25°C	F6

Sample	Fungal species	Loci used for	WGS accession no.	SRA	Medium and	Flig
name		identification <u>a</u>		accession no.	temperature <u>b</u>	no
F6_3S_1B_F	Aureobasidium pullulans	ITS	<u>JAGUQG000000000</u>	SRR14342123	PDA, 25°C	F6
F6_3S_1C_F	Aureobasidium pullulans	ITS	<u>JAGUQF000000000</u>	SRR14342122	PDA, 25°C	F6
F8_5S_2F	Cladosporium cladosporioides	TEF	<u>JAGUPV000000000</u>	SRR14342051	PDA, 25°C	F8
F8_5S_3F	Cladosporium cladosporioides	TEF	<u>JAGUPU000000000</u>	SRR14342048	PDA, 25°C	F8
F8_5S_4F	Cladosporium cladosporioides	TEF	JAGUPT0000000000	SRR14342047	PDA, 25°C	F8
F4_7S_F1_F	Cladosporium sphaerospermum	TEF	<u>JAHARS000000000</u>	SRR14342126	PDA, 25°C	F4
F8_4S_2B	Fusarium annulatum	TEF, RPB2	<u>JAHAPR000000000</u>	SRR14342059	R2A, 25°C	F8
F8_4S_3B	Fusarium annulatum	TEF, RPB2	<u>JAHAPP000000000</u>	SRR14342057	R2A, 25°C	F8
F8_4S_4P	Fusarium annulatum	TEF, RPB2	<u>JAHAPN000000000</u>	SRR14342055	BA, 37°C	F8
F8_4S_5P	Fusarium annulatum	TEF, RPB2	JAHAPL0000000000	SRR14342053	BA, 37°C	F8
F8_4S_1F	Fusarium annulatum	TEF, RPB2	<u>JAHAPT000000000</u>	SRR14342062	PDA, 25°C	F8
F5_8S_1A_F	Fusarium veterinarium	TEF	JAHARR000000000	SRR14342093	PDA, 25°C	F5
F5_8S_1B_F	Fusarium veterinarium	TEF	<u>JAHARQ000000000</u>	SRR14342082	PDA, 25°C	F5
F4_1A_F1_F	Penicillium citrinum	benA, CaM	<u>JAHART000000000</u>	SRR14342127	PDA, 25°C	F4
F5_1S_1A_F	Penicillium corylophilum	benA, CaM	JAGUQL0000000000	SRR14342115	PDA, 25°C	F5

Sample	Fungal species	Loci used for	WGS accession no.	SRA	Medium and	Flig
name		identification <u>a</u>		accession no.	temperature_	no
F5_1S_1B_F	Penicillium corylophilum	benA, CaM	JAGUQK000000000	<u>SRR14342104</u>	PDA, 25°C	F5
F6_4S_1A_F	Penicillium palitans	benA, CaM	<u>JAHARM000000000</u>	SRR14342121	PDA, 25°C	F6
F6_4S_1B_F	Penicillium palitans	benA, CaM	<u>JAHARL000000000</u>	SRR14342120	PDA, 25°C	F6
F6_4S_1C_F	Penicillium palitans	benA, CaM	<u>JAHARK000000000</u>	SRR14342119	PDA, 25°C	F6
F6_6S_1_F	Penicillium palitans	benA, CaM	JAHARA000000000	SRR14342107	PDA, 25°C	F6
F6_7S_1A_F	Penicillium palitans	benA, CaM	JAHAQR000000000	SRR14342097	PDA, 25°C	F6
F6_7S_1C_F	Penicillium palitans	benA, CaM	JAHAQQ000000000	SRR14342096	PDA, 25°C	F6
F6_8S_1A_F	Penicillium palitans	benA, CaM	<u>JAHAQI000000000</u>	SRR14342087	PDA, 25°C	F6
F6_8S_1C_F	Penicillium palitans	benA, CaM	JAHAQH000000000	SRR14342086	PDA, 25°C	F6
F8_6S-1F	Penicillium palitans	benA, CaM	<u>JAHAOZ000000000</u>	SRR14342036	PDA, 25°C	F8
F8_6S_2F	Penicillium palitans	benA, CaM	<u>JAHAPD000000000</u>	SRR14342041	PDA, 25°C	F8
F8_6S-3F	Penicillium palitans	benA, CaM	<u>JAHAOY000000000</u>	SRR14342035	PDA, 25°C	F8
F8_6S-4F	Penicillium palitans	benA, CaM	JAHAOX0000000000	SRR14342034	PDA, 25°C	F8
F8_6S_5F	Penicillium palitans	benA, CaM	<u>JAHAPC000000000</u>	<u>SRR14342040</u>	PDA, 25°C	F8
F8_6S_6F	Penicillium rubens	benA, CaM	JAHAPB0000000000	SRR14342039	PDA, 25°C	F8

Sample	Fungal species	Loci used for	WGS accession no.	SRA	Medium and	Flig
name		identification_a		accession no.	temperature <u>b</u>	no
F8_6S_7F	Penicillium rubens	benA, CaM	<u>JAHAPA000000000</u>	SRR14342037	PDA, 25°C	F8
F6_4S_B_2B	Rhodotorula mucilaginosa	ITS	<u>JAHARI000000000</u>	SRR14342116	R2A, 25°C	F6
F6_8S_B_1B	Rhodotorula mucilaginosa	ITS	<u>JAHAQG000000000</u>	SRR14342085	R2A, 25°C	F6
F6_8S_P_5A	Rhodotorula mucilaginosa	ITS	<u>JAHAQF000000000</u>	SRR14342081	BA, 37°C	F6
F6_8S_P_5B	Rhodotorula mucilaginosa	ITS	<u>JAHAQE000000000</u>	SRR14342080	BA, 37°C	F6
F6_8S_P_6A	Rhodotorula mucilaginosa	ITS	<u>JAHAQD000000000</u>	SRR14342079	BA, 37°C	F6
F6_8S_P_6B	Rhodotorula mucilaginosa	ITS	<u>JAHAQC000000000</u>	SRR14342078	BA, 37°C	F6
F8_5S_4P	Rhodotorula mucilaginosa	ITS	<u>JAHAPI000000000</u>	SRR14342046	BA, 37°C	F8
F8_5S_5P	Rhodotorula mucilaginosa	ITS	<u>JAHAPH000000000</u>	SRR14342045	BA, 37°C	F8
F8_5S_6P	Rhodotorula mucilaginosa	ITS	<u>JAHAPG000000000</u>	SRR14342044	BA, 37°C	F8
F6_1S_P_1A	Rhodotorula mucilaginosa	ITS	<u>JAHARP000000000</u>	SRR14342060	BA, 37°C	F6
F6_1S_P_1B	Rhodotorula mucilaginosa	ITS	<u>JAHARO000000000</u>	SRR14342049	BA, 37°C	F6
F6_1S_P_1C	Rhodotorula mucilaginosa	ITS	<u>JAHARN000000000</u>	SRR14342038	BA, 37°C	F6
F6_4S_B_2A		ITS	<u>JAHARJ000000000</u>	SRR14342117	R2A, 25°C	F6
F6_4S_B_2C		ITS	<u>JAHARH000000000</u>	SRR14342114	R2A, 25°C	F6

Sample	Fungal species	Loci used for	WGS accession no.	SRA	Medium and	Flig
name		identification_a		accession no.	temperature <u>b</u>	no
F6_4S_P_3B	Rhodotorula mucilaginosa	ITS	<u>JAHARG000000000</u>	SRR14342113	BA, 37°C	F6
F6_4S_P_3C	Rhodotorula mucilaginosa	ITS	<u>JAHARF000000000</u>	SRR14342112	BA, 37°C	F6
F6_4S_P_4A	Rhodotorula mucilaginosa	ITS	<u>JAHARE000000000</u>	SRR14342111	BA, 37°C	F6
F6_4S_P_4B	Rhodotorula mucilaginosa	ITS	JAHARD000000000	SRR14342110	BA, 37°C	F6
F6_4S_P_5A	Rhodotorula mucilaginosa	ITS	JAHARC0000000000	SRR14342109	BA, 37°C	F6
F6_4S_P_5B	Rhodotorula mucilaginosa	ITS	<u>JAHARB000000000</u>	SRR14342108	BA, 37°C	F6
F6_6S_B_1A	Rhodotorula mucilaginosa	ITS	<u>JAHAQZ000000000</u>	SRR14342106	R2A, 25°C	F6
F6_6S_B_1B	Rhodotorula mucilaginosa	ITS	<u>JAHAQY000000000</u>	SRR14342105	R2A, 25°C	F6
F6_6S_B_1C	Rhodotorula mucilaginosa	ITS	<u>JAHAQX000000000</u>	SRR14342103	R2A, 25°C	F6
F6_6S_P_1A		ITS	<u>JAHAQW00000000</u>	SRR14342102	BA, 37°C	F6
F6_6S_P_1B	Rhodotorula mucilaginosa	ITS	<u>JAHAQV000000000</u>	SRR14342101	BA, 37°C	F6
F6_6S_P_1C	Rhodotorula mucilaginosa	ITS	<u>JAHAQU000000000</u>	SRR14342100	BA, 37°C	F6
F6_6S_P_2A		ITS	<u>JAHAQT000000000</u>	SRR14342099	BA, 37°C	F6
F6_6S_P_2B		ITS	<u>JAHAQS000000000</u>	SRR14342098	BA, 37°C	F6
F6_7S_B_2A		ITS	<u>JAHAQP000000000</u>	SRR14342095	R2A, 25°C	F6

Sample	Fungal species	Loci used for	WGS accession no.	SRA	Medium and	Flig
name		identification <u>a</u>		accession no.	temperature <u>b</u>	no
F6_7S_B_2B	Rhodotorula mucilaginosa	ITS	<u>JAHAQO000000000</u>	<u>SRR14342094</u>	R2A, 25°C	F6
F6_7S_B_2C	Rhodotorula mucilaginosa	ITS	<u>JAHAQN000000000</u>	SRR14342092	R2A, 25°C	F6
F6_7S_P_6B	Rhodotorula mucilaginosa	ITS	<u>JAHAQM00000000</u>	<u>SRR14342091</u>	BA, 37°C	F6
F6_7S_P_7A	Rhodotorula mucilaginosa	ITS	<u>JAHAQL000000000</u>	SRR14342090	BA, 37°C	F6
F6_7S_P_7B	Rhodotorula mucilaginosa	ITS	JAHAQK000000000	SRR14342089	BA, 37°C	F6
F6_7S_P_7C	Rhodotorula mucilaginosa	ITS	<u>JAHAQJ000000000</u>	SRR14342088	BA, 37°C	F6
F8_1S_2B	Rhodotorula mucilaginosa	ITS	<u>JAHAQB000000000</u>	SRR14342070	R2A, 25°C	F8
F8_1S_3B	Rhodotorula mucilaginosa	ITS	<u>JAHAQA000000000</u>	SRR14342069	R2A, 25°C	F8
F8_3S_1B	Rhodotorula mucilaginosa	ITS	<u>JAHAPZ000000000</u>	SRR14342068	R2A, 25°C	F8
F8_3S_2B	Rhodotorula mucilaginosa	ITS	<u>JAHAPX000000000</u>	<u>SRR14342066</u>	R2A, 25°C	F8
F8_3S_3B	Rhodotorula mucilaginosa	ITS	<u>JAHAPV000000000</u>	SRR14342064	R2A, 25°C	F8
F8_4S_4B	Rhodotorula mucilaginosa	ITS	<u>JAHAPO000000000</u>	SRR14342056	R2A, 25°C	F8
F8_4S_5B	Rhodotorula mucilaginosa	ITS	<u>JAHAPM000000000</u>	SRR14342054	R2A, 25°C	F8
F8_5S_2B	Rhodotorula mucilaginosa	ITS	<u>JAHAPK000000000</u>	SRR14342052	R2A, 25°C	F8
F8_5S_3B	Rhodotorula mucilaginosa	ITS	<u>JAHAPJ000000000</u>	SRR14342050	R2A, 25°C	F8

Sample	Fungal species	Loci used for	WGS accession no.	SRA	Medium and	Flig
name		identification <u>a</u>		accession no.	temperature_	no
F8_6S_1B	Rhodotorula mucilaginosa	ITS	<u>JAHAPF000000000</u>	SRR14342043	R2A, 25°C	F8
F8_6S_2B	Rhodotorula mucilaginosa	ITS	<u>JAHAPE000000000</u>	SRR14342042	R2A, 25°C	F8
F8_3S_1P	Rhodotorula mucilaginosa	ITS	<u>JAHAPY000000000</u>	SRR14342067	BA, 37°C	F8
F8_3S_2P	Rhodotorula mucilaginosa	ITS	<u>JAHAPW000000000</u>	SRR14342065	BA, 37°C	F8
F8_3S_3P	Rhodotorula mucilaginosa	ITS	<u>JAHAPU000000000</u>	SRR14342063	BA, 37°C	F8
F8_4S_1P	Rhodotorula mucilaginosa	ITS	<u>JAHAPS000000000</u>	SRR14342061	BA, 37°C	F8
F8_4S_2P	Rhodotorula mucilaginosa	ITS	<u>JAHAPQ000000000</u>	SRR14342058	BA, 37°C	F8

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^abenA, β-tubulin; *CaM*, calmodulin; *RPB2*, DNA-directed RNA polymerase II subunit; *TEF*, translation elongation factor 1.

Genus-level identification was made via BLAST searches against the UNITE nuclear ribosomal internal transcribed spacer (ITS) database (34). Species identification was performed using specific loci suitable for species recognition (Table 1) (35). Homology searches were performed with extracted sequences against the NCBI nucleotide database and in-house Westerdijk Fungal Biodiversity Institute databases containing reference sequences; in case of doubt, identification was confirmed by constructing phylograms.

^bBA, blood agar; R2A, Reasoner's 2A agar; YPD, yeast extract-peptone-dextrose.

^cARED, advanced resistive exercise device; WHC, waste and hygiene compartment; PMM, permanent multipurpose module.

^dReference genome was not available; average sequencing depth was calculated from *k*-mer coverage.

Data availability.

The whole-genome sequences (WGSs) and raw data have been deposited in GenBank under BioProject accession number PRJNA723004. This project has also been deposited in the NASA GeneLab system (36) under project number GLDS-400. The version described in this paper is the first version.

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Associated Data

This section collects any data citations, data availability statements, or supplementary materials included in this article.

Data Availability Statement

The whole-genome sequences (WGSs) and raw data have been deposited in GenBank under BioProject accession number PRJNA723004. This project has also been deposited in the NASA GeneLab system (36) under project number GLDS-400 . The version described in this paper is the first version.

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